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Species

Trichodina cutcutiae sp. n. and *Trichodina cottidarum* Dogiel, 1948 (Ciliophora: Trichodinidae) from the freshwater fishes in the Baikka Beel of Moulvibazar district in Sylhet division, Bangladesh

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ABSTRACT

A survey was conducted on several species of wild freshwater fish fauna in the Baikka Beel, Moulvibazar district in Sylhet division between January and December 2015. During the time two species of *Trichodina* Ehrenberg, 1830 were recorded. A new species of *Trichodina* was found to infect the gills of *Leiodon cutcutia*, and *Trichodina cottidarum* Dogiel, 1948 was isolated from *Nandus nandus*. Based on the Klein's wet silver nitrate impregnation technique this new species is characterized by having medium sized body dimension with not darkly impregnated adhesive disc having several number of thick irregular patches; slim, slightly curved, cudgel-shaped blade with rounded distal margin above tangent point; smoothly curved anterior margin without prominent apex; large inter-blade space; well developed and thick blade connection; triangular and straight central part with sharply pointed tip and loosely fitted into following denticles; and slightly slanted ray in anterior direction, ending in rounded tip. The taxonomic and morphometric descriptions for the new species are presented along with rate of prevalence, infestation status, and morphometric and meristic data comparison with closely related species. Depending upon these morphological features and the unique shape and absence of variability of the denticles structure among the silver impregnated specimens of the present species, it may be said that to a lesser extent, it resembles *Trichodina fugu* Imai et al. 1997; and *Trichodina lascrucensis* Khan et al. 2008.

Key words: Ciliophora, Trichodinidae, *Trichodina cutcutiae* sp. n., *Trichodina cottidarum*, Bangladesh

1. INTRODUCTION

Trichodinids are parasitic ciliate, rarely symbiotic, invading the skin, fins and gills of pond reared and wild fishes (Dickerson 2006). These ciliates are widely distributed and infecting mainly or live as symbionts of aquatic chordates and non-chordates. The majority of trichodinids are described from freshwater environments (Arthur et al.1984a, 1984b; Lom 1970; Urawa et al. 1994; and Wellborn, 1967). To date approximately more than 400 species of *Trichodina* have been reported from different countries of the world (Wang et al. 2018).

In Bangladesh, studies on this group have been acquiring variant changes in several aspects through establishing four genera, *Trichodina* Ehrenberg, 1830; *Paratrichodina* Lom, 1963; *Tripartiella* Lom, 1959; and *Trichodinella* (Raabe, 1950) Šramek-Hušek, 1953 from various species of freshwater and estuarine edible fishes (Asmat et al. 1997, 2003a, b, c, 2004, 2005a, b, 2006, 2017; Bhouyain, 1999; Habib et al. 2008 and 2010; Kibria et al. 2009, 2010, 2011; Kibria and Asmat 2014; and Haque et al. 2018). The present investigation was carried out as a survey on the trichodinid ciliates of freshwater wild fish fauna from the Baikka Beel, Moulvibazar, Sylhet.

2. MATERIALS AND METHODS

During study time, fresh host fishes *Leiodon cutcutia* (4.0-7.0 cm × 8.0 - 12 gm) and *Nandus nandus* (8.0-10.0 cm × 15.0- 20 gm) were collected from local markets and adjacent areas of the Baikka Beel with the help of local people and fishermen. Gill smears were prepared on clean grease free slides and allowed to air-dry at the sampling sites. Air-dried gill's smear containing slides were transported to the laboratory and observed under a research microscope, OSK 9712 T-2 at 10x magnification due to sorting out slides with trichodinid ciliates. Slides positives for trichodinids were processed by using Klein's (1958) wet silver impregnation technique as recommended by Lom (1958). Each trichodinid population was photographed by using a SONY cyber shot camera in order to have comprehensive morphological data analyses and measurement.

Measurements of the trichodinid ciliates were done according to the uniform specific recommendation of Lom (1958), Wellborn (1967), Arthur and Lom (1984), and Van As and Basson (1989, 1992). All measurement data are given in micrometer and detailed

descriptions of the denticles presented in the accordance with the method proposed by Van As and Basson (1989). Minimum and maximum morphometric and meristic data are provided, range in parentheses by the arithmetic mean and standard deviation. For statistical analysis, morphometric measurements of 20 specimens for the species were considered.

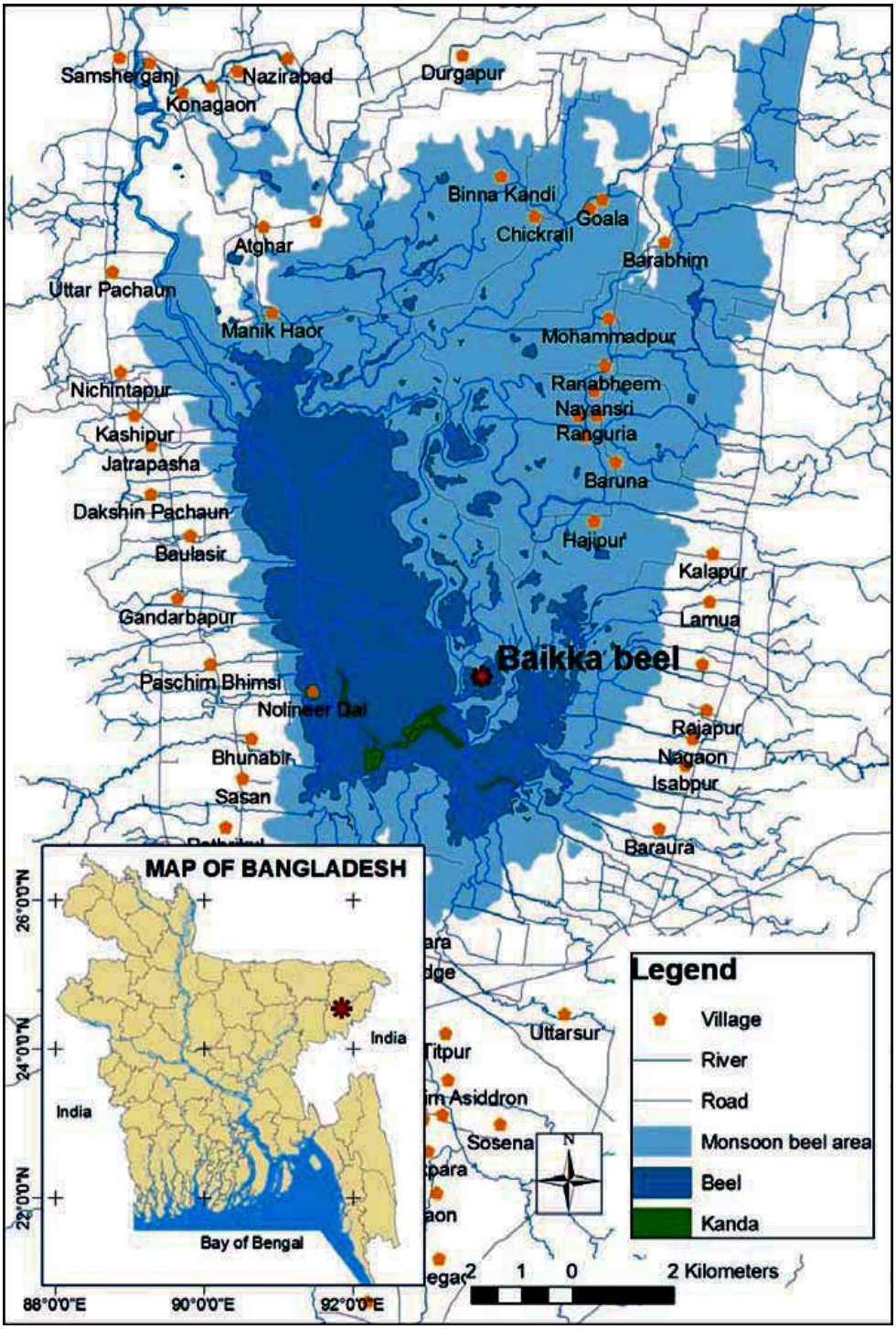


Figure 1 Map of sampling localities at the Baikka Beel, Bangladesh (Source: IPAC 2013, www. nishorgo. Org)

3. RESULTS AND DISCUSSION

***Trichodina cutcutiae* sp. n.** (Figs. 3, 4, 7-11; Table 1)

Description (n=20)**:** Medium sized ciliate. Saucer shaped body with diameter of 27.2-35.7 (32.3±2.6); concave adhesive disc, not darkly impregnated, presence of several thick irregular patches, 20.4-30.6 (27.3±2.6) in diameter; surrounded by finely striated

distinct border membrane 1.9-3.0 (2.6±0.3); central area medium, not well impregnated, dissimilar to the rest of the disc about 7.5-12.4 (10.1±2.7) in diameter; denticulate ring 13.8-20.4 (17.7±2.0); consisting of 24-27 (26.2±0.9) denticles with radial pins per denticle 6-8 (7.2±0.7); span and length 6.8-8.9 (8.1±0.7) and 2.9-4.1 (3.5±0.4) respectively; blade 2.4-4.6 (3.2 ± 0.5); length of ray 3.4-5.6 (4.1±0.5); central part 0.7-1.5 (1.0±0.3) in width. Adoral ciliary spiral could not be detected.

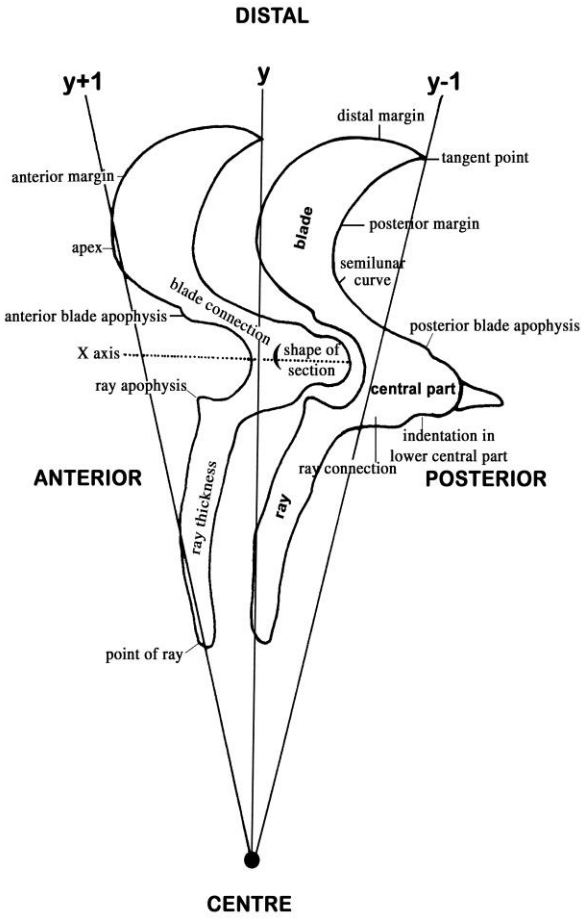


Figure 2 Denticle structure and construction of **X** and **Y** axes as fixed references for description denticles after Van As and Basson, 1989.

Denticle morphology: Blade slim, cudgel shaped, slightly curved and bulbous (Fig. 3). Distal margin of blade rounded, situated slightly higher than tangent point, prominently separate from anterior and posterior margin and also lying marginally far away from border membrane. Tangent point blunt, smooth and forms a small line rather than a point, situated slightly lower than distal margin. Anterior and posterior margin of blade more or less curved or smoothly angular without anterior blade apophysis. Lower border of apical depression sometimes impregnates with silver. Difficult to determine apex, present near to base of blade, never touches y+1 axis (Fig. 7). Posterior margin of blade having shallow, mark to any crescent curve. No prominent posterior blade apophysis. Large interblade gap existing between blades without interblade glistening particles. Blade connection thick. Central part delicate, triangular, straight or slightly curved, downwards with sharply pointed tip, extending more than half to y-1 axis and loosely fitted into preceding denticle. Indentation of lower central part not visible. Ray almost elongated, slightly slanted anteriorly. Ray larger than blade with post constriction part of ray to more extent, inflated, and bulbous with ending in rounded tip. Ray connection almost similar to blade connection. Ray tip touches y-1 axis. Lower half of the ray remains almost parallel to y axis. No visible central groove of ray.

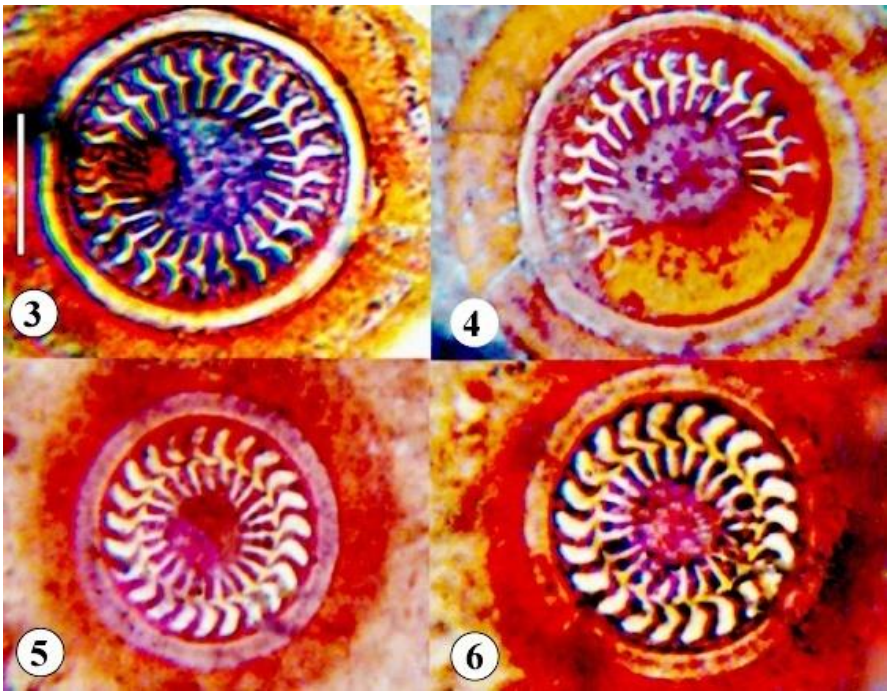


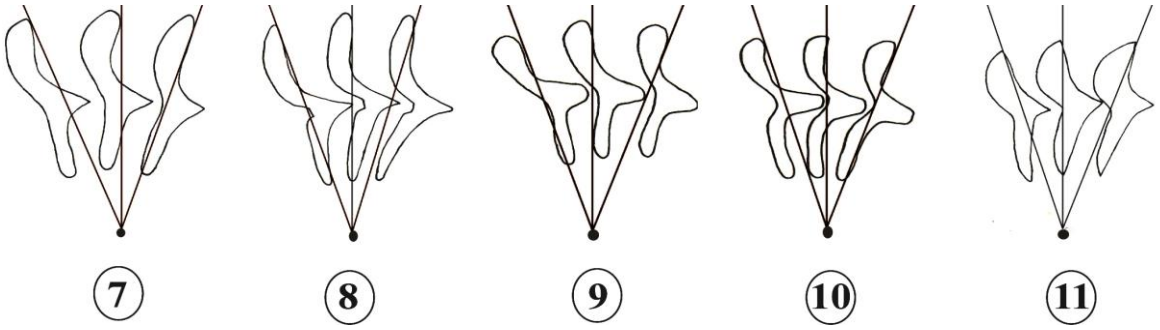
Figure 3-6. Photomicrographs of silver impregnated adhesive discs of *Trichodina cutcutiae* sp. n. (3-4), and *Trichodina cottidarum* (5-6). Scale bar 20 µm.

Table 1 Morphometric comparison of *T. cutcutiae* sp. n. with four populations of *Trichodina fugu* Imai et al. 1997; Xu, 2007; and Wang et al. 2015 and *Trichodina lascrucensis* Khan et al. 2008. Measurement in micrometer (µm)

Species	***T. fugu* (n=30)**	***T. fugu* (n=25)**	***T. fugu* (n=12)**	***T. fugu***	***T. lascrucensis***	***T. cutcutiae* sp. n. (n=20)**
Host	*T. rubripes*	*T. rubripes*	*T. rubripes*	*T. vermicularis*	*S. viridis*	*L. cutcutia*
Locality	Nagasaki, Japan	Shizuoka, Japan	Qingdao, China	Sanya, China	Coast of Central Chile at Las Cruces	Baikka Beel, Bangladesh
Location	Gills	Gills	Gills	Gills, body surface, fins, urogential sinus	Blood	Gills
References	Imai et al. (1997)	Imai et al. (1997)	Xu (2007)	Wang et al. (2015)	Khan et al. (2008)	Present paper
Diameter of body	36.0-60.0 (46)	50.0-64.0 (57.3)	33.0-44.0 (38.9±2.9)	33.0-54.0 (45.6±4.7)	60.0-84 .0 (70.8±5.1)	27.2-35.7 (32.3±2.6)
adhesive disc	28.0-48.0 (36)	39.0-54.0 (47.4)	29.0-39.0 (34.1±2.8)	29.0-49.0 (41.3±5.1)	48.0-60.0 (53.6±3.4)	20.4-30.6 (27.3±2.6)
denticulate ring	19.0-31.0 (24)	30.0-39.0 (32.6)	18.0-24.0 (21.3±1.8)	20.0-34.1 (28.8±3.5)	28.0-40.0 (33.0±3.2)	13.8-20.4 (17.7±2.0)
central area	-	-	-	-	-	0.5-12.4 (10.1±2.7)
Width of border membrane	1.0	1.0-3.0 (2.0)	2.0-3.0 (2.5±0.4)	0.9-2.8 (1.6±0.5)	2.0-3.0 (2.2±0.2)	1.9-3.0 (2.6±0.3)
Number of denticles	26-33 (29)	29-33 (31.6)	23-30 (27.3 ± 1.7)	24-30 (27)	30-36 (32.8 ± 1.1)	24-27 (26.2 ± 0.9)
radial pins/denticle	6-7	8	6-8	7-9	7-9 (8.0±0.4)	6-8 (7.2±0.7)
Span of denticle	-	-	7.5-9.5 (8.4±0.6)	9.5-15.0 (13.1±1.4)	-	6.8-8.9 (8.1±0.7)
Length of denticle	3.0-4.0	4.0-5.0 (4.3)	3.0-4.0 (3.5±0.4)	2.5-6.0 (4.5±0.8)	10.0-14.0 (11.8±1.1)	2.9-4.1 (3.5±0.4)
ray	3.0-4.5	4.0-5.5 (4.5)	2.5-3.5 (3.0±0.2)	3.0-6.5 (5.4±0.9)	2.0-4.0 (3.2±0.2)	3.4-5.6 (4.1±0.5)
blade	3.0-4.0	3.5-6.0 (4.5)	3.0-4.0 (3.5±0.4)	3.5-5.5 (4.8±0.5)	3.0-4.0 (3.4±0.3)	2.4-4.6 (3.2±0.5)
Width of central part	1.5-2.0	2.0-4.0 (3.0)	1.5-2.0 (1.8±0.2)	1.5-3.0 (2.2±0.4)	2.0-4.0 (3.2±0.3)	0.7-1.5 (1.0±0.3)
Degree of adoral ciliature	380°	380°	380-390°	400°	-	Could not be detected

The present populations of *T. cutcutiae* sp. n. were isolated from the gills of Ocellated pufferfish *L. cutcutia* at different seasons during study period. The individuals from all the examined host fishes exhibit a very little range of variability in case of denticle morphology and measurements. *T. cutcutiae* sp. n. can be characterized by having medium sized body dimension without darkly impregnated adhesive disc, presence of several thickly irregular patches; slim, slightly curved, cudgel-shaped blade with rounded

distal margin above tangent point; smoothly curved anterior margin without prominent apex; large inter-blade space; well developed and thick blade connection; triangular and straight central part with sharply pointed tip and loosely fitted into following denticles; and ray slightly slanted in anterior direction, ending in rounded tip.



Figures 7-11 Diagrammatic drawings of the denticles of *Tichodina* species: 7. *Trichodina cutcutiae* sp. n. from the gills of *Leiodon cutcutia* in Bangladesh; 8. *T. fugu* from the gills of *Takifugu rubripes* in Japan, redrawn from Imai et al. 1997; 9. from the gills of *T. rubripes* in China, redrawn from Xu, 2007; 10. from the Gills, body surface, urogential sinus of *T. vermicularis* in China, redrawn from Wang et al. 2015; and 11. *T. lascrucensis* from the blood of the *Scartichthys virdis* in Chile, redrawn from Khan et al. 2008.

In terms of denticle morphology of the silver impregnated adhesive disc the presently described trichodinid exhibits closeness to *T. fugu* Imai et al. (1997) and *T. lascrucensis* Khan et al. (2008).

T. fugu was first described by Imai et al. (1997) from the gills of the one year old juvenile Tiger puffer *Takifugu rubripes* in Nagasaki and Shizuoka prefectures, Japan. *T. fugu* was also reported by Xu (2007) as a country record for China (Qingdoa, the yellow Sea, China) from the gills of same marine fish *T. rubripes*. Xu (2007) re-described the population of *T. fugu* showed very close morphometric resemblance to original specimens of Imai et al. (1997). Moreover, Wang et al. (2015) also reported *T. fugu* from the gills of wild marine fish *T. vermicularis* in Sanya (the South Sea, China). Imai et al. (1997) described species *T. fugu* by using formaldehyde fixed cells for Klein's dry silver impregnation, which made the adhesive disc of the species was not well impregnated, however the number of the slender and characterized rod shaped denticles provide a space for easy studying, as also observed by Xu (2007) and Wang et al. (2015).

The denticle morphology of presently described ciliate appears similar to *T. fugu* as described by Imai et al. (1997). In both species, denticle slim; rounded distal margin; blunt and smooth tangent point; and uniformly impregnated adhesive disc. The presently described species can clearly be distinguished from *T. fugu* by considering a number of crucial characteristic features. In *T. fugu*, (i) centre of adhesive disc darkly impregnated but contains non-impregnated granular particles (vs lightly impregnated, having several thick irregular patches in place of granular particles); (ii) denticle of the blade almost straight (vs blade slightly curved); (iii) distal margin of blade lying very close to the border membrane (vs lying far away from the inner side of the border membrane); (iv) interblade space small (vs large interblade space); (v) the central part ">" shaped, downwardly directed and tightly fitting into the preceding denticles (vs triangular, straight with sharply pointed and loosely fitting into the preceding denticles); (vi) ray extremely slender, almost straight with blunt point (vs stout, slightly slanted in anterior direction, inflated, ending in bulbous or rounded tip); (vii) body dimension large, 46.0 µm (vs small, 32.3 µm); and (viii) number of denticles 26-33 (vs number of the denticles 24-27).

Xu (2007) and Wang et al. (2015) reported specimens of *T. fugu* from China are distinguished from Imai et al. (1997) described species *T. fugu* in the such aspects like body dimension, blade shape, distal margin of blade, posterior projection, blade connection, shape of central part etc. Xu (2007) re-described specimens of *T. fugu* from the gills of Tiger pufferfish *T. rubripes* from mariculture ponds on the China coast of Yellow Sea. The presently described species was obtained the gills of Ocellated pufferfish *L. cutcutia*. The two species are similar in the general appearance of the blade shape. However, presently described species can be clearly distinguished from Xu (2007) in many aspects. In *T. fugu* of Xu (2007) (i) body dimension considerably larger, 38.9µm (vs smaller, 32.3 µm); (ii) tip of blade slightly thicker (vs thinner); (iii) anterior and posterior margin of the blade straight and almost parallel to each other (vs slightly curved); (iv) posterior projection of the blade present (vs no posterior projection of the blade); (v) central part comparatively thick with rounded tip, fitting tightly into the preceding denticles and extending to the y-1 axis (vs triangular shaped, straight with sharply pointed tip, loosely fitting into the preceding denticles and extending more than half to the y-1 axis); and (vii) ray slightly shorter than the blade (vs blade slightly shorter than the ray).

Wang et al. (2015) recorded *T. fugu* also shows remarkable variation in body dimension and denticle morphology from the presently described species. In Wang et al. (2015) re-described specimens of *T. fugu* (i) body dimension large, 45.64 µm (vs small, 32.3 µm); (ii) span of the denticles large, 13.09 µm (vs small, 8.1 µm); (iii) distal margin of the blade lying very close to border membrane (vs distal margin of the blade lying marginally far away from border membrane); (iv) straight anterior and posterior margin of the blade, nearly parallel to each other (vs slightly curved anterior and posterior margin of the blade, not parallel to each other); and (v) comparably robust , downwardly directed central part with the rounded tip and fitting tightly into the preceding denticles (vs central part straight, triangular with sharply pointed tip and loosely fitted into the preceding denticles).

Trichodina lascrucensis was established by Khan et al. (2008) from blood of *Scartichthys viridis* from coast of Central Chile at Las Cruces. Notwithstanding the denticle of presently described trichodinid ciliate exhibits some similarities in case of the blade shape and the ray to *T. lascrucensis*. However, the body diameter of *T. lascrucensis* is the largest than presently described trichodinid ciliate. In contrast, the adhesive disc dimension, the denticular ring, the number of the denticles, the radial pins per denticle, length of the denticle, shape and width of the central part also mostly variable from presently described trichodinid ciliate.

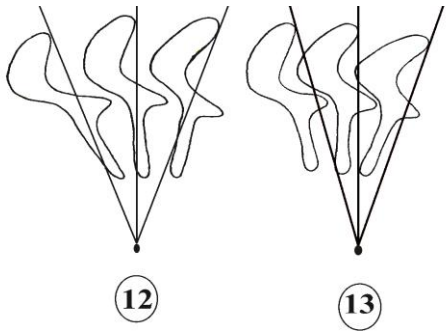
Twenty five out of 30 (83.33%) of the examined *L. cutcutia* were discovered as infected with *T. cutcutiae* sp. n. The level of infection rate was higher. The highest number of ciliates was observed invading the gills of the hosts in the month of January 2015.

Type Host: *Leiodon cutcutia* Hamilton, 1822. **Type Locality**: The Baikka Beel (24.3514° N, 91.6979° E) of Moulvibazar district in Sylhet division, Bangladesh. **Type Location on host:** Gills. **Type specimens:** Holotype, slide CUZM-TC-BB-1 on 09 February 2015; Paratypes, slide CUZM-TC-BB-2 on 09 February 2015 is in the collection of the Museum of Department of Zoology, University of Chittagong-4331, Chittagong, Bangladesh. **Etymology**. Named after the specific name of the host fish species, *Leiodon cutcutia*.

***Trichodina cottidarum* Dogiel, 1948** (Figs. 5, 6, 12-13; Table 2)

Host: *Nandus nandus* Hamilton, 1822. **Locality**: The Baikka Beel (24.3514° N, 91.6979° E) of Moulvibazar district in Sylhet division, Bangladesh. **Location on host:** Gills. **Prevalence**: Twenty Eight out of fifty specimens examined (56.00%). **Infection**: Medium. **Voucher specimens:** Two slides (CUZM-TCD-1-BB and CUZM-TCD-2-BB) with sliver impregnated specimens prepared on 10th January 2015 have been deposited in the Museum of Department of Zoology, University of Chittagong, Chittagong 4331, Chittagong, Bangladesh.

Description (n=20)**:** Small sized trichodinid ciliate 27.2-38.9 (32.4±3.7) in diameter. Adhesive disc rounded, rarely oblong, 21.3-28.1 (25.1±2.0) surrounded by finely striated border membrane 2.9-3.9 (3.4±0.3) wide. Central area uniformly well impregnated and almost similar to rest of adhesive disc, 7.8-10.5 (8.8±0.8) diameter. Denticulate 13.1-23.8 (17.5±2.9), consisting of 22-25 (23.1±1.1) with radial pins per denticle, 5-7 (6.0±0.6) per denticle. Span and length of denticle 7.8-8.7 (8.1±0.3) and 2.9-3.5 (3.0±0.2) respectively. Length of ray 2.5-3.7 (3.0±0.3), blade 3.4-4.4 (3.9±0.2), and width of central part 1.2-1.6 (1.4±0.1).



Figures 12-13 Diagrammatic drawings of the denticles of *Tichodina* species: 12. *Trichodina cottidarum* from the gills of *Nandus nandus* in Bangladesh; 13. *T. cottidarum* from *Ceraocottus* (Cottidae) in Japan Sea, redrawn from Dogiel, 1948

Denticle morphology: Blade narrow, delicate, curved, almost sickle shaped and filling almost entire space between y and y+1 axes (Fig. 5). Distal margin slightly angular, distinguishable from anterior margin and lying closely associated with border membrane. Tangent point blunt, slightly upper than distal margin. Anterior margin smoothly curves down and forms shallow rounded apex, never touching y+1 axis. Apical depression not visible and without anterior blade projection. Posterior margin forms a deep crescent like depression and deepest point of the crescentic depression lying below apex of blade. Blade connection thin. Central part delicate, triangular, downwardly directed with sharp pointed tip, firmly fitted into following denticles, and extending more than halfway to y-1 axis (Fig. 12). Section of central part above and below similar. Ray straight or slightly slanted anteriorly with equal

thickness throughout the length and ending with blunt point of tip. Ray slightly shorter than blade, almost parallel to y axis without distinct central groove. Tip of ray almost touches or crosses y-1 axes. Ray connection thick and ray apophysis absent.

Trichodina cottidarum was originally established by Dogiel, 1940 as *T. domerguei* f. *borealis*, whereby this form was supposed to occur in the host species *Myoxocephalus quadricornis* and *Pleuronectes* in the White Sea. Dogiel (1948) also recorded this trichodinid ciliate from the Gulf of Peter the Great (Sea of Japan). It has several forms viz., *T. cottidarum* f. *cottidarum* Dogiel, 1940 from Cottidae and Agonidae, *T. cottidarum* f. *hemitripteri* Dogiel (1940) from *Hemitripterus* (Scorpaenidae) and *Ceraocottus* (Cottidae), and *T. cottidarum* f. *alcichthys* Dogiel (1940) from *Alcichthys*. In 1951, Poljansky differentiated further these forms the Barents Sea, *T. cottidarum* f. *barenzi* from Cottidae and *T. cottidarum* f. *cyclopteri* from *Cyclopterus lumpus*. In 1954, Stein reported *T. cottidarum* f. *marisalbi* from *Enchelyopus cimbrius* (Gadiformes) from the Baltic Sea.

Table 2. Morphometric comparison of *Trichodina cottidarum* obtained in the present study with *Trichodina cottidarum* Dogiel, 1940; Dogiel, 1948, and Raabe, 1958. Measurement in micrometer (µm).

Species	***T. cottidarum***	***T. cottidarum***	***T. cottidarum***	***T. cottidarum* (n=20)**
Host	*M. quadricornis, Pleuronectes*	Cottidae, Agonidae	*Cottus scorpius*	*N. nandus*
Locality	White Sea	Japan Sea	Baltic Sea	Baikka Beel,Bangladesh
Location				Gills
Reference	Dogiel (1940)	Dogiel (1948)	Raabe (1958)	Present paper
Diameter of			31.0-45.0 (40)	
body	38.0-45.0 (41)	33.0-39.0	30.0-45.0 (40)	27.2-38.9 (32.4±3.7)
adhesive disc	25.0-37.0	17.0-27.0	17.5-24.0 (21)	21.3-28.1 (25.1±2.0)
denticulate ring	-	12.6-19.0	-	13.1-23.8 (17.5±2.9)
central area	-	-	-	7.8-10.5 (8.8±0.8)
Width of border membrane	-	-	-	2.9-3.9 (3.4±0.3)
Number of denticles	26-28 (22)	20-22 18-29	22-23 20-25	22-25 (23.1±1.1)
radial pins/denticle	-	3--2	7-8	5-7 (6.0±0.6)
Span of denticle	-	-		7.8-8.7 (8.1±0.3)
Length of denticle	-	-	-	2.9-3.5 (3.0±0.2)
ray	-	-	-	2.5-3.7 (3.0±0.3)
blade	-	-	-	3.4-4.4 (3.9 ± 0.2)
Width of central part	-	-		1.2-1.6 (1.4 ± 0.1)
Degree of adoral ciliature	-	-	-	Could not be detected

Dogiel (1948) discussed the problems of differentiating the species *T. cottidarum* draws attention to the location of micronucleus in this form on the elongation of the arm of the macronucleus and not in its concavity as it appears in the form from Pleuronectus.

The problem of the separation of *T. cottidarum* Dogiel, 1948 seems to be clear, but none of the Russian authors present a satisfactory picture of morphological difference. Which would differentiate this species form other species, particularly no description the silver adsorbing system, so important for the taxonomic diagnosis was given. All the authors attempted only to show the biometric differences. Which are more or less distinctive and very various in the various forms of *T. cottidarum* Dogiel, 1940, and which are even more divergent in the individual forms of *T. domerguei*. Later, Raabe (1959) recorded and presented neat and clean drawing of *T. cottidarum* from the gills *Cottus scorpius* from the Baltic Sea.

In the course of the present study period, *T. cottidarum* was recorded as new country record for Bangladesh. Presently discussed specimens were found to invade the gills of *N. nandus* have coincident morphological features of that originally described species by Dogiel (1940). However, the presently discussed specimen is slightly different from originally described species. Body dimension, adhesive disc, denticle ring, number of denticles, and number of radial pins per denticle show a range of variation. Body dimension (means 32.4) of present specimen is smaller than those described by Dogiel, 1940 (means 41); Dogiel, 1948 (means 36); Stryjecka-Trembaczowska, 1953 (means 36); Poljansky, 1951 (means 36); and Raabe, 1958 (means 40). These variations are distinguishable as diversification of hosts, and various geographical locations.

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a. Conception of research work;
b. Study design and methods;
c. Data acquisition and collation from research field;
d. Analysis and interpretation of data;
e. Writing the manuscript of research paper;
f. Critical revision of the manuscript of research paper;
g. Drawing and mapping;

Name of authors	Contribution (a-g)	Contribution to the total (%)
Md. Amdadul Haque	e & c	28.57
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